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EVALUATION OF ANTIFUNGAL PROPERTY AND PHYTOCHEMICAL ANALYSIS OF UNDEREXPLOITED LEAFY VEGETABLES

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ABSTRACT: The adverse environmental impact of fungicides used in plant disease control is escalating day by day. Generally, plant diseases are controlled by synthetic fungicides, chemical methods, and bio-control agents. Chemical methods lead to deterioration of soil fertility and cause pollution. Being highly stable and resistant to biodegradation, chemical fungicides also enter the food chain and are responsible for many harmful effects on human health and the environment. Hence, eco-friendly alternative methods are of great urgency. One of the prominent approaches in this direction includes utilizing plant extracts with natural antifungal constituents. So, a study was attempted to determine the antifungal and phytochemical activity of underexploited leafy vegetables. Methanol and water extracts of the leaves were tested against two economically important plant pathogens *Alternaria solani* and *Pythium aphanidermatum* and the antifungal activity of plant extracts was determined. All the plant species under study showed inhibitory activity against the plant pathogens. However, *B. diffusa* and *C. frutescens* exhibited maximized efficiencies. Moreover, the water extracts of *B. diffusa* and *C. frutescens* (1%) were tested against *Phytophthora* fruit rot of Brinjal under open field conditions. Reduced disease severity was observed in *C. frutescens* water extract. Phytochemical analysis revealed that alkaloids, carbohydrates and flavonoids are the active components present in the water and methanol leaf extracts; among them, saponins, tannins, alkaloids, flavonoids and terpenoids are major components responsible for antifungal activity. Results of the present study indicate that plant extracts can be effectively used as a botanical fungicide as an alternative management method to tackle plant disease.

INTRODUCTION: The annual crop losses of the world as a result of plant diseases have been increasing day by day, in which fungal disease contributes a major share [1]. Existing control measures are not enough to deal with the emergence of outbreaks of plant fungal diseases.

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Chemical methods lead to deterioration of soil fertility and cause pollution. Being highly stable and resistant to biodegradation, chemical fungicides also enter the food chain and are responsible for many harmful effects on human health and the environment.

Therefore, the focus is shifting towards alternative strategies for the control of fungal diseases of plants. Based on the knowledge that plants have recently developed their defense against fungal pathogens [2] in different parts of the world, attention has been paid to the exploitation of higher plant products as novel chemotherapeutics in plant protection. Similarly, traditional medicinal knowledge on the use of plant extracts for the treatment of fungal infection has also increased the interest of researchers all over the world for developing an alternative control strategy to reduce dependency on synthetic fungicides. Many workers have tried extracts of medicinal plants for plant disease management, but they are costly and less available. However, the demand for economically viable and environmentally safe strategies for plant disease control has amplified the use of botanical fungicides. In this context, biodegradable materials like fresh plant leaf extracts serve as suitable alternatives.

Plants can produce secondary metabolites like phenols, phenolic acids, quinones, flavones, flavonoids flavonols, tannins, coumarins, carvacrol, eugenol, and thymol which were noted to be highly active against the pathogen. These groups of compounds exhibit antimicrobial activity and serve as plant defense mechanisms against pathogenic microorganisms. Vegetables contain a wide variety of biologically active, non-nutritive compounds known as phytochemicals [3]. Antifungal substances obtained from plants have no side effect on the environment, thus giving a significant advantage. So, we mainly consider the leaf of underexploited vegetables, which serve as suitable alternatives for fungal infection. These are inexpensive and widely available compared to medicinal plants.

Antifungal agents based on natural products have always been promising in the control of fungi. Moreover, these agents are not toxic and are decomposed easily. Complete elimination of chemical fungicides for controlling plant diseases in modern agriculture may be impossible, but a logical reduction in their application is feasible. There is no doubt that the use of biological control agents is one of the safest solutions for disease control and it leads us towards a sustainable agricultural system in near future. The battle to protect plant health is ongoing, and plant disease management is essential for our continued ability to feed a growing human population. Numerous works of literature have highlighted the ability of the under-exploited vegetables to control fungal diseases [4, 5, 6] effectively. Among them, some of the underexploited leafy vegetables include *Boerhavia diffusa*, *Capsicum frutescens*, *Cnidoscolus aconitifolius*, *Sauropus androgynus*, *Talinum portulacifolium*, *Moringa oleifera*, *Basella alba*, *Pisonia grandis*, *Fleurya interrupta*, *Coccinia grandis*. In this light, the present study is an attempt to investigate the phytochemical and antifungal effects of selected underexploited leafy vegetables using two important plant pathogens namely *Alternaria solani* and *Pythium aphanidermatum* test organisms.

MATERIALS AND METHODS: Healthy, disease-free plant leaves were collected separately and brought to the laboratory, and washed thoroughly under the tap water to remove dirt. The leaves were then dried under shade for two to three days until the weight was reduced to half. Extract the leaf (10%) was prepared by soaking 1.5 g of powdered dry leaves in 15 ml distilled water or methanol. It was then mixed thoroughly and kept undisturbed for 24 hrs at room temperature. The supernatant was filtered through Whatman No. 1 filter paper, and the filtrate was preserved. Accelerated solvent extraction was also carried out.

***In-vitro* Evaluation by Poisoned Food Technique:** The sterilized, poisoned PDA was melted and cooled to 40 °C. PDA devoid of the leaf extract but with the same amount of distilled water or methanol was plated to serve as the control. Mycelial disc (5 mm) taken from the seven-day-old culture of *P. aphanidermatum* and *A. solani* was placed on the center of the plates and incubated at 27+/-2 °C. Mycelial growth was measured daily till there was full growth in control. The percent inhibition (PI) was calculated using the formula given by Vincent [7]:

$$PI = C - T \times 100/ T$$

PI = percent inhibition, C= Growth of the pathogen in control (mm), T = Growth of pathogen in treatment (mm).

***In-vivo* Evaluation of Leaf Extracts against *Phytophthora* Fruit rot of Brinjal:** The field experiment for managing fruit rot of brinjal caused by phytophthora sps was carried out superimposing the treatment on an existing brinjal field with Surya variety. The experimentation was designed using randomized block design and with three treatments and seven replications, in which each plot consists of three Brinjal. The spacing between each plant is

60 cm × 45 cm. The experiment was carried out from August to October 2019.

Preliminary Phytochemical Screening of Vegetable Leaves: Phytochemical analysis of water and methanol extracts was conducted to identify the presence of carbohydrates, alkaloids, saponins, phenol, tannins, sterols, proteins, reducing sugar, terpenoids [8] and flavonoids [9].

RESULTS: The leaves were dried at 50 °C to constant dry weight. Fresh weight and percent of the dry weight of leaves were calculated. The percent dry weight varies from seven to 50 percent, and it was highest in *M. oleifera* and the lowest in *B. alba*. Leaves with more water content showed less percent of dry weight.

The percent dry weight of leaves such as *B. diffusa, C. frutescens, C. aconitifolius, S. androgynus, T. portulacifolium, P. grandis, F. interrupta* and *C. grandis* varies between nine to 34 per cent. The moisture content of leaves has a direct relationship with antifungal property [10]. In the present study highest moisture content was observed in *B. alba* followed by *T. portulacifolium*.

***In-vitro* Evaluation of Leaf Extracts using Poison Food Technique:** All plant species showed significant inhibitory activities in both water and methanol extracts. However, *B. diffusa* and *C. frutescens* were noted to be superior. In water extract, percent inhibition varied from 84 (*B.* diffusa) to 0 (*F. interrupta* and *S. androgynous*) against *P. aphanidermatum*, and in the case of *A. solani*, more than 80 percent inhibition was exhibited by leaf extract of *T. portulacifolium*, *C. frutescens*, *C. grandis*, *B. alba*, *P. grandis* and *B. diffusa*. Whereas, the cent per cent inhibition of *P. aphanidermatum* was recorded in methanol extract of *C. frutescens*, *C. grandis*, *T. portulacifolium*, *B. alba*, *P. grandis* and *F. interrupta*. In the case of *A. solani* more than 80 per cent inhibition was recorded by methanol extract of *T. portulacifolium, S. androgynous, M. oleifera, C. frutescens, C. grandis, B. alba, P. grandis, C. aconitifolius* and *B. diffusa.* The results are depicted in **Fig. 1** and **2.**

Leaves of plants that showed more than 80 per cent inhibition in water and methanol extracts *viz., C. frutescens, C. grandis, B.alba* and *T. portulacifolium* were used for accelerated solvent extraction study. When subjected to accelerated solvent extraction, in methanol, the per cent inhibition of *A. solani* increased slightly (up to 87 per cent) in *T. portulacifolium*.

However, in the case of *C. frutescens*, the per cent inhibition decreased to 41 against *A. solani* and there was no inhibition in *P. aphanidermatum.* The reduction in inhibition may be due to the degradation of some of the secondary metabolites in high temperatures (200 psi), to which samples were exposed during the extraction. The details are furnished in **Fig. 3.** Accelerated solvent extraction offers a better alternative to several thermal extraction methods in terms of less solvent consumption, shorter operational time, high recoveries, good reproducibility, cost-effectiveness and minimal sample manipulation for the extraction process.

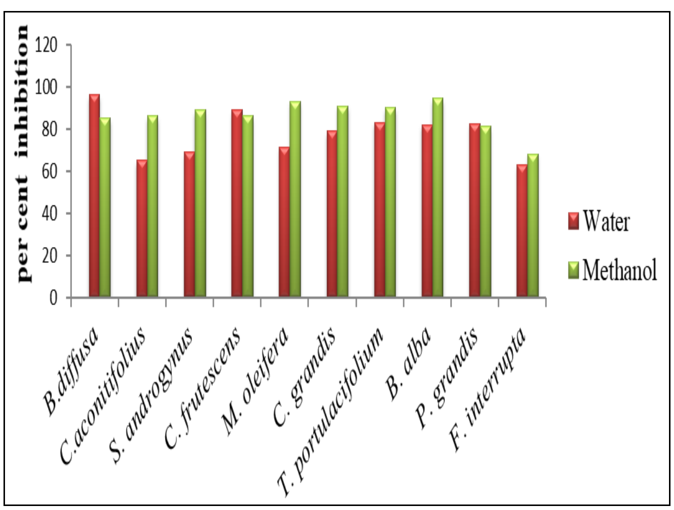

FIG. 1: *IN-VITRO* EVALUATION OF LEAF EXTRACTS AGAINST *ALTERNARIA SOLANI*

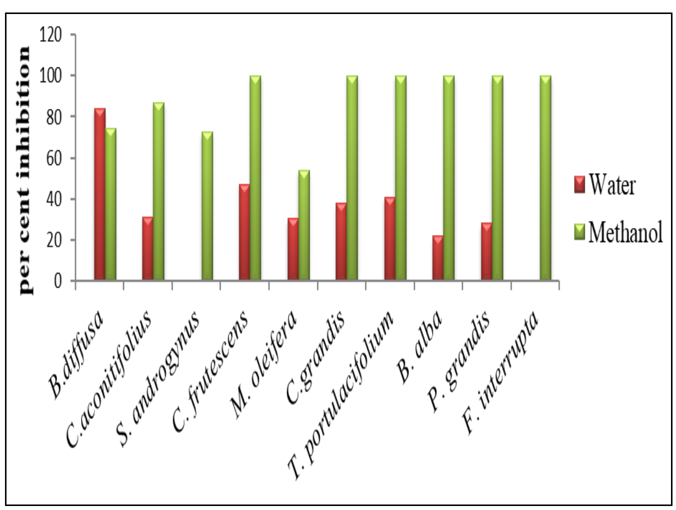

FIG. 2: *IN-VITRO* EVALUATION OF LEAF EXTRACTS AGAINST *PYTHIUM APHANIDERMATUM*

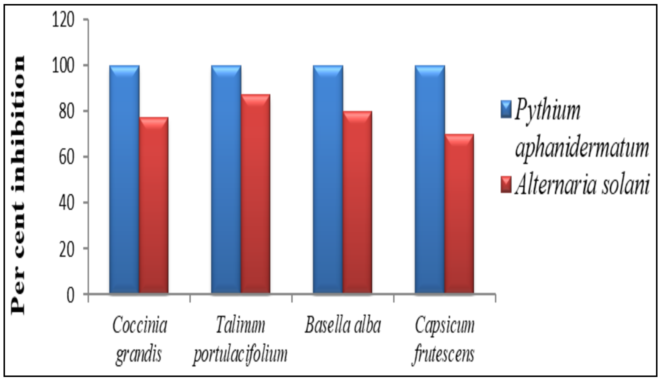

FIG. 3: *IN-VITRO* EVALUATION OF ACCELERATED SOLVENT EXTRACT OF VEGETABLE LEAVES AGAINST *PYTHIUM APHANIDERMATUM* AND *ALTERNARIA SOLANI* IN METHANOL EXTRACT

***In-vivo* Evaluation of Leaf Extracts against *Phytophthora* Fruit Rot of Brinjal:** The water extracts of *B. diffusa* and *C. frutescens* (1%) were tested against *Phytophthora* fruit rot of Brinjal under open field conditions. The lowest disease severity was observed in *C. frutescens* water extract; this was followed by *B. diffusa*. The details are furnished in **Fig. 4.**

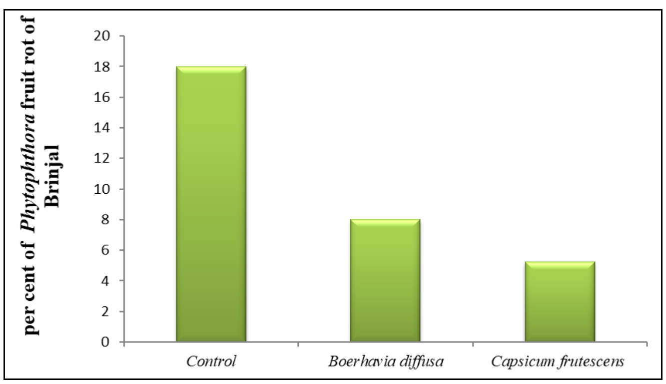

FIG. 4: EFFECT OF PLANT EXTRACTS ON *PHYTOPHTHORA* FRUIT ROT OF BRINJAL

Preliminary phytochemical analysis of vegetable leaves: The selected ten vegetable plants are the source of the secondary metabolites *i.e.*, alkaloids, flavonoids, terpenoids and reducing sugars. Leafy vegetables play a vital role in preventing various diseases. Leafy vegetables play a vital role in preventing various diseases. The antidiuretic, anti-inflammatory, antianalgesic, anticancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal activities are due to the presence of the above-mentioned secondary metabolites. Carbohydrates, alkaloids and flavonoids are the common compounds present in all plants. Among them, saponins, tannins, alkaloids, flavonoids and terpenoids are major components responsible for antifungal activity shown in **Tables 1 and 2**. Water extraction of *C. grandis* contain Carbohydrate (+++), protein (+), amino acid (+), Flavonoid (++), Alkaloids (+++) and steroids (++). Whereas in methanol extract it contains carbohydrate (+++), protein (+), amino acids (+), alkaloids (+++), flavonoids (++) and steroids (++) are present. Carbohydrate and alkaloids are higher in both water and methanol extracts, while amino acids and proteins are only seen in methanol extract. Kaviya and Shukla [11] found that carbohydrates were absent in methanol extract, and water phenol, tannin, and saponins were present. In water and methanol extract of *C. frutescens*, the concentration of carbohydrate (+++), alkaloids (+++), flavonoids (+++) and steroids (+++) are higher. Saponin (+) is only present in methanol extract of *C. frutescens*. The most abundant and pungent constituents of chilies, capsaicinoid, belong to the alkaloid class. Gurnani *et al*. [12] found that alkaloids, flavonoids, polyphenols, steroids, and tannins were observed in methanolic extract of *C. frutescens*. A similar result was found in Koffi-Nevry *et al*. [13] and Vinayaka *et al*. [14]. Roa *et al*. [15] found that in *C. annuum*, flavonoids, saponins, tannins, and phenols were absent.

T. portulacifolium contain carbohydrate (+++), protein (+), flavonoids (++), alkaloids (+++), steroids (++), phenol (+), tannins (+) and saponins (++) in water extracts. Phenols and tannin were only present in water extracts of *T. portulacifolium*. Saponin was moderately present in *T. portulacifolium* were as in *C. frutescens, B. alba, S. androgynous* and *F. interrupta* extracts their presence is very low in concentration. Whereas in methanol extract carbohydrate (++), alkaloids (++), flavonoids (+), and steroids (+) are mainly present. Aja [16] showed the same result that *T. portulacifolium* possesses alkaloids, flavonoids, phenol, tannin, and saponin in the water extract. In *C. aconitifolius* carbohydrate (++), alkaloids (++), flavonoids (++), steroids (++), and triterpenoids (+) are the main compounds existing in methanol extract.

In water extract, it contains the same component as in methanol, but their concentration was high they are carbohydrate (+++) and alkaloids (+++), flavonoids (+++), steroids (+++) the additional compound are protein (++) and amino acid (+)

while triterpenoids were absent. Flavonoids are the main component in *C.aconitifolius* leaves extract exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, analgesic, anti-allergic, cytostatic, and antioxidant properties [17].

In water extraction of *B.alba* it contain carbohydrate (+++), alkaloids (+++), flavonoids (++), steroids (++) and saponnins (+). *B. alba* is only one species that contain triterpenoids (++), but in methanol extract, it is present but in moderate concentration. In methanol extract it contain carbohydrate (+), alkaloids (+++), flavonoids (++), steroids (++) and triterpenoids (++). Murakami [18] and Maisuthisakul [19] reveal that *B. alba* contains basellasaponins and peptide, phenolic compounds.

In *M. oleifera*, carbohydrates (+) and alkaloid (+) are the compound present in methanol extract. But in water extracts, the carbohydrate (+++) alkaloid (+++) and flavonoid (++) are mainly present.

In leaf extract of *S. androgynous* alkaloids (+++), saponins (+), steroids (++), carbohydrates (+), and flavonoids (++) are major compounds in the water extract. The same result was found by Hegde and Divya [20]. In methanol extract, the component was the same as in water, but their concentration was low.

In *F. interrupta* the amount of secondary metabolite is very low *i.e.*, in methanol, it contains only carbohydrate (+) and alkaloids (++), whereas in water, it contains carbohydrate (+), alkaloids (+++), flavonoids (++), steroids (+++) and saponins (+) were present. Thamizh Selvam *et al*. [21] found the same result that the amount of alkaloids is very low in methanol extract.

Water extract of *P. alba* contain carbohydrate (++), alkaloids (++), flavonoids (++), steroids (+). Whereas protein, amino acid, phenol, tannins, triterpenoids and saponins remain completely absent.

In methanol extract, it contains only carbohydrate (+), flavonoids (+) and steroids (+). Poongothai and Shubashini [22] have reported that the methanol extract of *P. alba* leaf possesses flavonoids and resins, but tannins, alkaloids, terpenoids, glycosides, saponins, and proteins were absent.

In *B. diffusa* water extract, the key component was carbohydrate (+), protein (+), amino acids (+), alkaloids (+++), flavonoids (+), triterpenoids (+) and steroids (++). A vital component is the presence of alkaloids. The results were different in the case of methanol extract it comprises only the presence of carbohydrate (++) all others are absent.

TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF VEGETABLE LEAVES USING WATER EXTRACT

S. no.	Phytochemical analysis	Name of plants									
		Coccinia grandis	***Capsicum frutescens***	***Talinium portulacifolium***	***Cindoscols aconitifolius***	***Basella alba***	***Moringa oleifera***	***Sauropus androgynous***	***Fleurya interrupta***	***Pisonia grandis***	***Boerhavia diffusa***
		Carbohydrates									
1	Molisch's test	+++	+++	+++	+++	+++	++	+	+	+	+
	Fehling test	+	+	+	+	+	+	+	+	+	+
	Benedict's test	+	+++	+	++	++	+	+	+	++	+
	Iodine test	-	-	-	-	-	-	-	-	-	-
2		**Protein**									
	Biuret test	+	-	+	++	-	-	-	-	-	+
	Xanthoproteic test	+	-	+	++	-	-	-	-	-	+
3		**Amino Acids**									
	Ninhydrin test	+	-	-	+	-	-	-	-	-	+
4		**Alkaloids**									
	Mayer's test	++	++	++	++	++	++	++	+	+	+
	Wagner test	+++	+++	+++	+++	+++	+++	+++	+++++		+++
5		**Flavonoids**									
	NaOH test	++	+++	++	++	+	+	+	+	+	+
	H_2SO_4 test	++	+++	++	+++	++	++	++	++	++	+
6		**Phenols & Tannins**									
	Folin test	-	-	+	-	-	-	-	-	-	-
	Acetic acid	-	-	+	-	-	-	-	-	-	-
7		**Triterpenoids**									
	Salkowski test	-	-	-	-	++	-	-	-	-	+
8		**Steroid**									
	Salkowski test	++	+++	++	+++	++	-	+++	++	+	++
9		**Saponins**									
	Foam test	-	+	++	-	+	-	+	+	-	-

* '+ 'low, '++' average, '+++'high, '–' nil

TABLE 2: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF VEGETABLE LEAVES USING METHANOL EXTRACT

S. no.	Phytochemical analysis	Name of plants									
		Coccinia grandis	*Capsicum frutescens*	*Talinium portulacifolium*	*Cindoscols aconitifolius*	*Basella alba*	*Moringa oleifera*	*Sauropus androgynous*	*Fleurya interrupta*	*Pisonia grandis*	*Boerhavia diffusa*
	Carbohydrates										
1	Molisch's test	+++	+++	++	++	+	+	+	+	+	++
	Fehling test	+	+	+	+	+	+	+	+	+	+
	Benedict's test	+	+++	+	+	+	+	+	+	+	+
	Iodine test	-	-	-	+	-	-	-	-	-	-
2	**Protein**										
	Biuret test	+	-	-	-	-	-	-	-	-	-
	Xanthoproteic test	+	-	-	-	-	-	-	-	-	-
3	**Amino Acids**										
	Ninhydrin test	+	-	-	-	-	-	-	-	-	-
4	**Alkaloids**										
	Mayer's test	++	++	++	++	++	+	+	+	-	-
	Wagner test	+++	+++	+	++	+++	+	+	++		-
5	**Flavonoids**										
	NaOH test	++	+++	+	+	+	-	+	-	+	-
	H_2SO_4 test	++	+++	+	++	++	-	+	-	+	-
6	**Phenols & Tannins**										
	FolinTest	-	-	-	-	-	-	-	-	-	-
	Acetic acid	-	-	-	-	-	-	-	-	-	-
7	**Triterpenoids**										
	Salkowski test	-	-	-	+	++	-	-	-	-	-
8	**Steroid**										
	Salkowski test	++	+++	+	++	++	-	+	-	+	-
9	**Saponins**										
	Foam test	-	+	-	-	-	-	-	-	-	-

* '+ 'low, '++' average, '+++'high, ' – ' nil

DISCUSSION: Results of the study suggest that leaf extracts of vegetables can be potential botanical fungicides. The organic solvent dissolves more in bioactive compounds and makes it available for antifungal action, as reflected by the enhanced inhibition of pathogens by methanol extract compared to water extract. The laboratory observations could be reflected under field conditions as well.

The major bioactive components in vegetable leaf extract belong to alkaloids, flavonoids, steroids, and saponins. Phytochemical analysis revealed that antifungal action by plant secondary metabolites is rather complex. Variable efficacy recorded by accelerated solvent extracts may be due to the variable heat sensitivity of the biochemical compounds present in leaves.

There is a correlation between the kind and content of secondary metabolites and the antifungal action of the leaf extract. However, further studies and analyses are needed to decipher the specific and synergistic effects of different subgroups of compounds and to identify novel compounds and activities.

CONCLUSION: There were several reports regarding utilizing plant-based products as alternatives for insecticides; however, their application as fungicides is scanty. Complete elimination of chemical fungicides for controlling plant diseases in modern agriculture may be impossible, but a logical reduction in their application is feasible. There is no doubt that botanical fungicides from the leaves of vegetables could be the cheapest and safest solution against plant diseases shortly.

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